

CLAIMS

1. (Original) A multiplexed assay for monitoring the level of transcription of one or more genes in response to one or more potential regulatory stimuli, comprising:

placing transfected cells in each of a plurality of wells, where the cells in each well are transfected with a genetic construct comprising a selected promoter operatively linked to the coding sequence for an enzyme having a selected enzymatic activity;

adding to the cells in each well a probe selected from a set of probes, where each probe in the set is cleavable by the enzyme into a substrate moiety and an electrophoretic tag (e-tag) reporter having a detection group and a separation modifier that confers on the e-tag reporter, a unique electrophoretic mobility with respect to the e-tag reporters derived from the other probes in the set;

incubating the cells and associated probes while exposing the cells to a potential regulatory stimulus;

obtaining the tags from the cells;

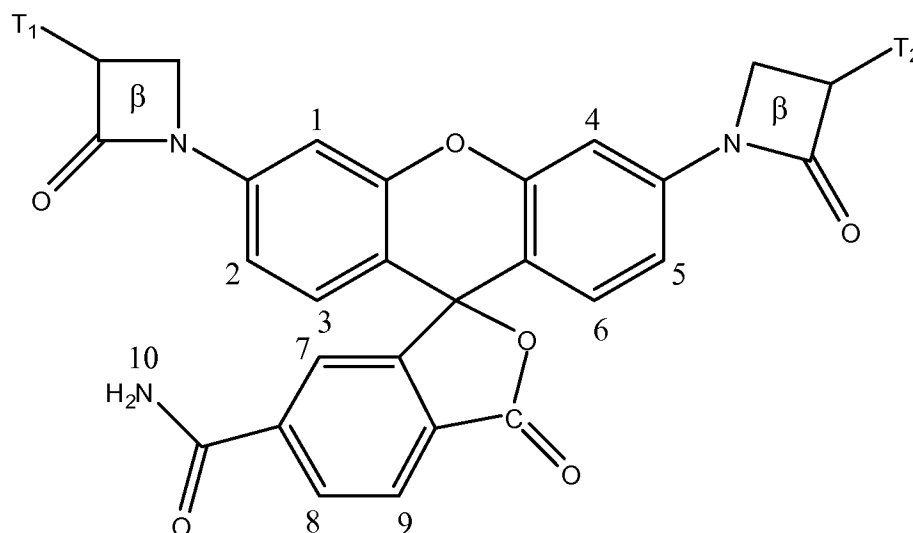
electrophoretically separating the combined tags; and

determining, from the electrophoretic mobility and level of detection group of each separated e-tag reporter, the level of transcriptional response of each cell to the potential regulatory stimulus to which the cells were exposed.

2. (Original) The method of claim 1, wherein said incubating is carried out in separate wells, and said obtaining includes combining the cells from separate wells, and obtaining said tags from combined wells.
3. (Original) The method of claim 2, wherein the potential regulatory stimuli include one or more test compounds, and said exposing includes adding the test compound(s) to the individual cell-containing wells.

4. (Original) The method of claim 3, for monitoring the dose response to a single test compound, with respect to the level of transcription of a selected gene under the control of a selected promoter, wherein the cells in each well are transfected with a construct comprising said promoter operatively linked to said coding sequence, and said incubating includes adding a different concentration of the test compound to each of a plurality of the wells.
5. (Original) The method of claim 3, for monitoring the response to each of a plurality of different test compounds, with respect to the level of transcription of a selected gene under the control of a selected promoter, wherein the cells in each well are transfected with a construct comprising said promoter operatively linked to said coding sequence, and said incubating includes adding a different test compound to each of a plurality of the wells.
6. (Original) The method of claim 1, for monitoring the response to a single test compound of each of a plurality of genes under the control of each of a plurality of different promoters, with respect to the level of transcription of the genes, wherein the cells in each of a plurality of wells are transfected with a different construct comprising one of said promoters operatively linked to said coding sequence, and said incubating includes adding the test compound to each of the plurality of the wells.
7. (Previously presented) The method of claim 1, wherein said incubating is carried out in a single well containing a plurality of different cells, each containing a different probe, and said obtaining includes obtaining released tags from the well.
8. (Original) The method of claim 7, for monitoring the response to a single test compound of each of a plurality of genes under the control of each of a plurality of different promoters, with respect to the level of transcription of the genes, wherein the different cells in said well are each transfected with one of a plurality of different constructs, each comprising one of said promoters operatively linked to said coding sequence.
9. (Original) The method of claim 1, wherein the enzyme is selected from the group consisting of β -lactamase, β -galactosidase, and esterase, a protease, and a nuclease.

10. (Original) The method of claim 1, wherein each probe in the set further comprises a transport moiety that facilitates transport of the probe into a cell, and a transport moiety linkage that is subject to cleavage within the cell to release the transport moiety and thereby inhibit probe transport of the probe out of the cell.
11. (Original) The method of claim 10, wherein said enzyme is β -lactamase, and one or more of the probes of the set has the form:



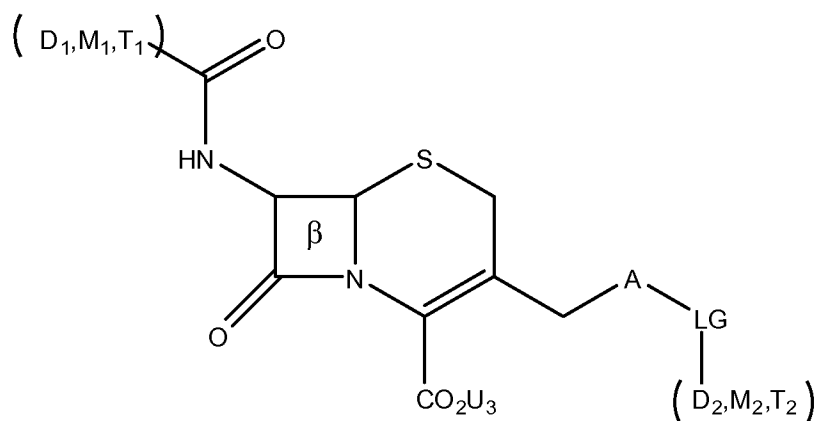
where exemplary positions of transport moieties are shown as T₁, T₂ and T₃;

a substrate for the enzyme, S, is exemplified as a four-member β -lactam ring, labeled β ;

the main part of the structure comprises a fluorescein derivative, wherein various positions are numbered that are subject to chemical modification; and

a separation modifier, M, (not shown) is present at one of the numbered positions.

12. (Previously presented) The method of claim 10, wherein said enzyme is β -lactamase, and one or more of the probes of the set has the form:



where exemplary positions of transport moieties are shown as T_1 and T_2 ;

exemplary positions of detection groups are shown as D_1 and D_2 ;

exemplary positions of separation modifiers are shown as M_1 , and M_2 ; and

a substrate, S , for the enzyme is exemplified as a four-member β -lactam ring, labeled β ,
wherein cleavage of the ring induces electron flow towards the acceptor, shown as A ,
causing cleavage of the probe by the elimination of the leaving group, shown as LG .

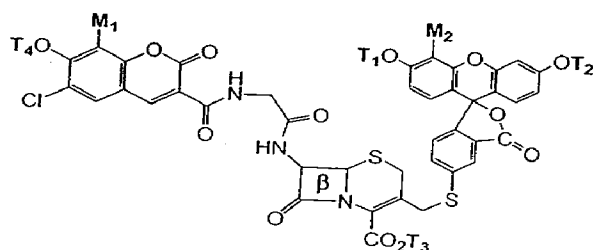
13. (Original) The method of claim 12, wherein

the probes comprise fluorophores at positions D_1 and D_2 ;

the two fluorophores are capable of exhibiting efficient fluorescence resonance energy transfer; and

cleavage of the β -lactam ring results in separation of the two fluorophores, thereby restoring fluorescent emission from the shorter wavelength fluorophore.

14. (Original) The method of claim 13, wherein one or more of the probes of the set have the form:



where exemplary positions of the transport moiety are shown as T₁, T₂, T₃ and T₄;

the first detection group D₁ is exemplified as cephalosporin;

the second detection group D₂ is exemplified as a fluorescein;

exemplary positions of the separation modifier are shown as M₁ and M₂; and

the substrate is exemplified as a four-member β -lactam ring, labeled β .

15. (Original) The method of claim of claim 1, wherein said detection group is a fluorescent moiety.

16. (Original) The method of claim 1, wherein said detection group includes a catalytic moiety capable of catalyzing a detectable reaction.

17. (Previously presented) The method of claim 1, wherein the probes in the set have the form (D, M_j) -S, where

(D, M_j) is the detection group D linked to the separation modifier M_j having a unique separation characteristic for each probe j in the set;

S is a substrate for the enzyme; and

the action of the enzyme on the probe produces an e-tag reporter of the form (D, M) -S', where S' is a residue of the substrate remaining with the e-tag reporter after reaction of S with the enzyme.

18. (Previously presented) The method of claim 1, for determining the extent of interaction of a first hybrid protein having a DNA-binding domain that binds to the selected

promoter, and a first interaction domain; and a second hybrid protein having a transcriptional activation domain and a second interaction domain that is to be tested for interaction with the first interaction domain; where (i) said promoter is capable of activation by a polypeptide having a transcriptional activation domain when the transcriptional activation domain is in sufficient proximity to the gene, (ii) said cells contains said first and second hybrid proteins, and (iii) said determining includes determining the extent of interaction of the two hybrid proteins by the level of e-tag reporter determined.

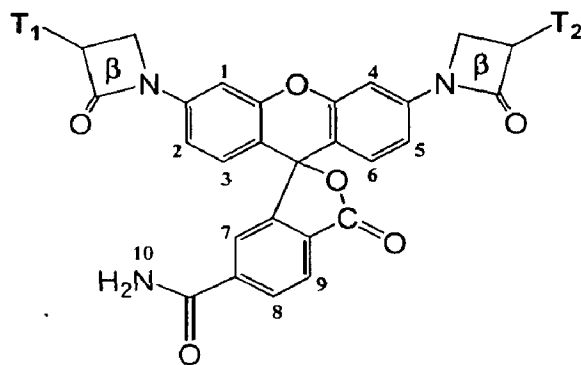
19. (Original) The method of claim 18, for use in screening a test compound for an effect on interaction between the first and second hybrid proteins, which further includes adding the test compound to the cells and determining the amount of e-tag reporter produced, and from that, the extent to which the test compound has an effect on binding between the two hybrid proteins.
20. (Original) The method of claim 18, wherein the promoter is a repressible promoter, and the cells further contain a repressor gene construct comprising an inducible promoter operatively linked to the coding sequence for a protein capable of binding to and repressing said repressible promoter.
21. (Original) The method of claim 20, for use in screening a test compound for an effect on interaction between the first and second hybrid proteins, which further includes adding the test compound to the cells and determining the amount of e-tag reporter produced, and from that, the extent to which the test compound has an effect on binding between the two hybrid proteins.
22. (Original) The method of claim 1, for determining the extent of binding of a hybrid protein to a designated DNA sequence, said hybrid protein having a transcriptional activation domain fused to a DNA-binding domain, said designated DNA sequence being operatively linked to the selected promoter, where (i) the promoter is capable of activation by a polypeptide having the transcriptional activation domain when the transcriptional activation domain is in sufficient proximity to the promoter, (ii) the cells

contain the hybrid protein, and (iii) said determining includes determining the extent of binding of the hybrid protein to the designated DNA sequence by the level of e-tag reporter determined.

23. (Original) The method of claim 22, for use in screening a test compound for an effect on interaction between a hybrid protein and a designated DNA sequence, which further includes adding the test compound to the cells after the mixing step, and comparing the determined amount of each separated reporter to the amount determined from the cells that were not exposed to the test compound, thereby determining the effect of the compound on interaction between any of the hybrid protein and the designated DNA sequence.

24-27 (Canceled)

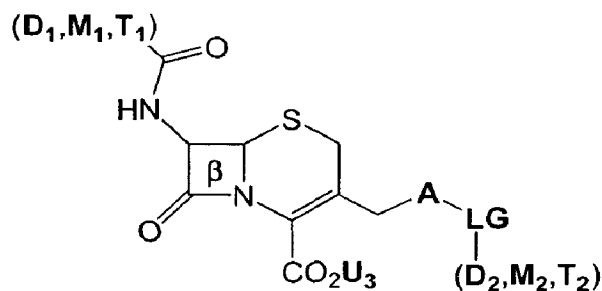
28. (Withdrawn) The composition of claim 27, wherein one or more of the probes of the set has the form:



where exemplary positions of transport moieties are shown as T₁ and T₂;
a substrate for the enzyme, S, is exemplified as a four-member β-lactam ring, labeled β;

the main part of the structure comprises a fluorescein derivative, wherein various positions are numbered that are subject to chemical modification; and
a separation modifier M_j (not shown) is present at one of the numbered positions.

29. (Withdrawn) The composition of claim 27, wherein one or more of the probes of the set have the form:



where exemplary positions of transport moieties are shown as T₁, T₂ and T₃;

exemplary positions of detection groups are shown as D₁ and D₂;

exemplary positions of separation modifiers are shown as M₁ and M₂; .

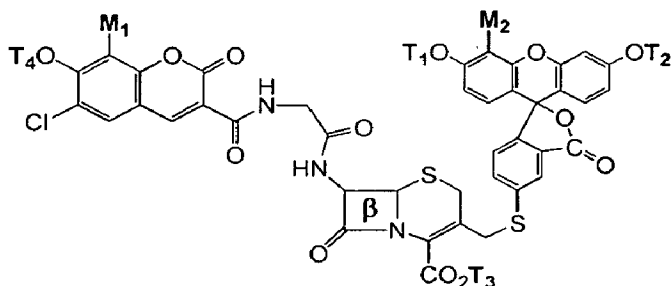
and

a substrate for the enzyme, S, is exemplified as a four-member β -lactam ring, labeled β , wherein cleavage of the ring induces electron flow towards the acceptor, shown as A, causing cleavage of the probe by elimination of the leaving group, shown as LG.

30. (Withdrawn) The composition of claim 29, wherein
the probes comprise fluorophores at positions D₁ and D₂;
the two fluorophores are capable of exhibiting efficient fluorescence resonance energy transfer; and

cleavage of the β -lactam ring results in separation of the two fluorophores, thereby restoring fluorescent emission from the shorter wavelength fluorophor.

31. (Withdrawn) The composition of claim 30, wherein one or more of the probes of the set have the form:



where exemplary positions of the transport moiety are shown as T₁, T₂, T₃ and T₄;
the first detection group D₁ is exemplified as a cephalosporin;
the second detection group D₂ is exemplified as a fluorescein;
exemplary positions of the separation modifiers are shown as M₁ and M₂; and
the substrate is exemplified as a four-member β -lactam ring, labeled β .